REMARKS

This is in response to the Official Action of September 9, 2003, and the telephone interview among Examiner Cheu, Examiner Le, Dr. Agris, and Mr. Sibley of February 18, 2004.

Additionally this is in response to the Notice of June 17, 2004, in which correction of the phosphate group to the originally shown structure was requested. This has been done above.

I. Restriction requirement.

The rejoinder if group I, claims 1-13, with group II, claims 14-18, in the previous official action is acknowledged with appreciation. Claims 19-22 are cancelled herein in view of the restriction requirement of record, without prejudice to the filing of a divisional application thereon.

II. Inventorship.

As discussed during the interview, the listing of the inventors appearing after the title in the application has been amended to recite Paul F. Agris alone, the inventor of the claims currently of record.

III. Double patenting.

This rejection being provisional, applicant will defer action. It is noted that the present case is expected to issue prior to the cited case (serial no. 10/190795), and that any double patenting issues may accordingly be resolved in the cited case.

IV. Claim objections.

The informality in claim1 has been corrected in the amendment above: "a organic protecting group" has been amended to read "an organic protecting group." It is respectfully submitted that this objection may be withdrawn.

V. Indefiniteness rejection.

Claims 2-6 and 56-60 stand rejected as indefinite, it being noted that the structures do not recite an oxygen consistent with the term "nucleotide" as used elsewhere in the specification. As discussed at the interview, the claims have been amended to clarify that nucleotides are contemplated, consistent with the definition of nucleotide in the specification (see, e.g., paragraph 4 on page 7) A copy of the pages A. Lenhinger, cited in the specification at page 7 paragraph 4, is enclosed herewith to further demonstrate that this is a typographical error, as suggested by the Examiner. It is noted that the presence of oxygen is consistent with the usage of the terms "oligomer" in the specification (see paragraph 3 on page 7), consistent with the discussion of oligonucleotides in the paragraph beginning at the bottom of page 11 of the specification, and consistent with the oligonucleotides actually used in the Examples (see, *e.g.,* Example 1) Accordingly, it is respectfully submitted that this constitutes correction of a typographical error which would be readily apparent to skilled persons, and that this rejection may now be withdrawn.

Conforming amendments to Formula I have been made in the specification.

VI. The Prior Art.

Claims 1-3, 7-18, 56-57 and 61-63 stand rejected as anticipated by US Patent No. 5464759 to Lewis et al. While applicants respectfully disagree for the reasons set forth herein, to narrow the issues and expedite allowance of this case, claims 2-3 and 56-57 have been cancelled, without prejudice, and the protected nucleotides recited in dependent claims 4-6 and 58-60 have been incorporated into independent claims 1 and 14. Applicants note for the record that the phrase "one of said nucleotides" when referring to a protected nucleotide in the claims is to be considered open-ended, and does not preclude the possibility that other nucleotides in the oligonucleotide may also be protected.

For for foregoing reasons, it is respectfully submitted that this rejection should be withdrawn.

Further, as discussed in part at the interview, Lewis et al. deals with permanent capping groups and not temporary protecting groups.

it is noted that high-quality chemical synthesis of antisense oligonucleotides via nucleobase and sugar-protected phosphoramidites is crucial to the expectations of low toxicity, reduced side effects, and low costs (Dove, A. (2002) *Nature Biotechnology* **20**, 121-124). However, neither the coupling reaction producing the growing polymer chain nor the subsequent deprotection of the full-length oligonucleotide occurs with 100% efficiency (Gilar, M. (2001) *Analytical Biochemistry* **298**, 196-206; Fu, C., Smith, S., Simkins, S.G. and Agris, P.F. (2002) Identification and quantification of protecting groups remaining in commercial oligonucleotide products using monoclonal antibodies. *Analytical Biochemistry* **306,** 135-143). Thus, quality and regulatory concerns about antisense therapeutics were expressed early by scientists at the Food and Drug Administration (FDA; Rockville, MD) (Kambhampati, R.V., Chiu, Y.Y., Chen, C.W. & Blumenstein, J.J. (1993) *Antisense Res. Dev.* **3**, 405-410). Purification by length (gel electrophoresis, size exclusion chromatography, or by antibody against a terminal **capping** group; ie. Lewis, et al.) generates full length molecules, but does not exclude incomplete deprotection. Incomplete deprotection of nucleoside-reactive groups could be responsible for the unexplained results observed in the early *in vitro* and cellular stages, and the immunological response observed in clinical trial stages of drug discovery.

The monomer units used in chemical synthesis of oligonucleotide (or for that matter in any directed polymer synthesis) have "protecting groups" rather than "capping groups" (Scaringe SA, Francklyn C, Usman N. (1990) *Nucleic Acids Res.* **18,** 5433-41; Gasparutto D, Livache T, Bazin H, Duplaa AM, Guy A, Khorlin A, Molko D, Roget A, Teoule R. (1992) *Nucleic Acids Res.* **20,** 5159-66.). Protecting groups protect reactive functional groups (for instance, exocyclic amines on A, G and C and the 2'OH on ribonucleotides) except for the one functional group used to extend the polymer. Thus, site-specific protecting groups enable experimental control of the direction of the polymer synthesis. These protecting groups are chemically distinct to covalently bind to the nucleosides specific reactive functional group. Their most important property is the **temporary nature** of the covalent bond to the nucleosides reactive functional group. The protecting groups are stable during automated chemical synthesis of the polymer

as each additional monomer is coupled. After coupling of each nucleotide at 98-99% efficiency, the 1-2% of the polymer chains that have not received the next monomer are capped. However, the protecting groups are removeable when the polymer synthesis is complete. Removing or deprotection is not 100% complete, as discussed further below.

As discussed in the interview, capping groups, in contrast to protecting groups, are permanent rather than temporary, and are permanent in the Lewis et al. reference.

As discussed in the declaration of Dr. Agris submitted concurrently herewith, mass spectrometry and capillary electrophoresis are used to detect aborted sequences of oligonucleotides and gel electrophoresis and chromatography (HPLC) to purify full-length oligomer. But these methods do not identify and quantify incompletely deprotected oligonucleotides or purify completely deprotected oligonucleotides from incompletely deprotected oligonucleotides. To address this problem, monoclonal antibodies (MAbs), ELISA, and dot-blot assays were developed by the inventor for the specific identification and quantification of the commonly used nucleic acid base- and sugar-protecting groups: benzoyl, isobutyryl, isopropylphenoxyacetyl, and dimethoxytrityl. As shown in the declaration, using the MAb dot-blot assay, 5 of 16 commercial full-length DNA products obtained from eight different companies were found to have 1.0–5.2% of the benzoyl and isopropylphenoxyacetyl protecting groups remaining.

As further discussed in the declaration of Dr. Agris, each MAb was capable of reproducibly and uniquely detecting 8–32 pmol of the respectively protected nucleoside in an intact DNA or RNA sample composed of 320–640 nmol of the deprotected nucleoside. In a direct comparison, HPLC nucleoside composition analysis of enzyme-hydrolyzed DNA was limited to the detection of 2–5 nmol of protected nucleoside. Each MAb characteristically recognizes the distinctive protecting group no matter the compound to which the protecting group is attached, DNA, RNA, cyclodextrin. Thus, MAbs selectively identify and quantify picomoles of remaining protecting groups on antisense therapeutics and oligonucleotide diagnostics.

During the interview the Examiners suggested that the submission of a Rule 132 declaration would be helpful in the instant case, and such a submission is made concurrently herewith.

During the interview the Examiners further suggested that it would be helpful to describe the process by which the protected oligonucleotides utilized in the instantly claimed methods are made. Claim 14 has been so amended above. Support for this amendment is found throughout the specification, including the discussion of protecting groups and deprotection at pages 7-8 (particularly the paragraph bridging pages 7-8).

VII. Conclusion.

The points and concerns raised in the outstanding Office Action having been addressed in full, it is submitted that this application is in condition for allowance, which action is respectfully requested.

Respectfully submitted,

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Previous Enclosures submitted March 9:

Lehninger pages

Agris Declaration